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Identification and mapping of a major gene (Ft1) involved in fructification time in the interspecific cross Coffea pseudozanguebariae × C. liberica var. Dewevrei: impact on caffeine content and seed weight

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Abstract Fructification time was studied in the interspecific cross *Coffea pseudozanguebariae* × *C. liberica* var. *Dewevrei* (PSE × DEW). Parental species, F_1 hybrids and offspring of the first backcross generation (BC_1), consisting of F_1 × PSE (BCPSE) and F_1 × DEW (BCDEW) plants, were observed. Fructification time can be split into two independent visual phases: the full-growth period, from blooming up to the end of fruit growth, and the maturation phase, defined by the green to red color change. Fructification time was found to be an additive trait. The full-growth period showed a bimodal distribution in the BCDEW hybrid, suggesting the involvement of *Ft1*, a major gene that was mapped on linkage group E. The main effects of *Ft1* were to lower caffeine content and 100-seed weight, without any impact on chlorogenic acid, trigonelline and sucrose contents. Two molecular markers were identified that bracket *Ft1* and which could be used for early marker-assisted selection.

Keywords *Coffea* · Fructification time · Major gene · QTL · Caffeine · Seed weight

Introduction

In *Coffea*, fructification time (FT) ranges from 2 to 11 months (Dussert et al. 2000). There is a geographical gradient that separates East and West African species, with eastern species generally having the lowest FT. At 2.3 months, *C. pseudozanguebariae* exhibits one of the shortest FTs. In contrast, canephoroids (*C. canephora* Pierre, *C. congensis* Froehner) and liberico-excelsoid species (*C. liberica* Hiern var. *liberica* Hiern and var. *Dewevrei* de Wild. et Dur.) have the longest FTs (10–11 months). This gradient overlaps the caffeine and chlorogenic acid (CGA) content gradients (Anthony et al. 1993), suggesting that long FT could produce high caffeine and CGA[1] contents and that these products could result from an accumulation process. There is a similar colinearity between FT and seed weight, the latter an agronomic trait of economic importance for the coffee market.

Breeding for FT could indirectly improve green bean quality. Indeed, a lower FT could reduce coffee bitterness through a decrease in caffeine and CGA contents. This could also make it possible to adjust the harvest date to obtain optimal climate conditions with respect moisture avoidance. However, a decrease in FT is only interesting when there is no negative impact on seed weight. An interspecific cross between *C. pseudozanguebariae* Bridson (PSE) and *C. liberica* var. *Dewevrei* (DEW), whose F_1 and BC_1 generations were evaluated for biochemical traits (Barre et al. 1998; Ky et al. 1999, 2000a, 2001), constitutes a basic material for such investigations.

The results described in this paper are presented in four parts: (1) a descriptive and quantitative analysis of FT, including the main characteristics (mean and range) of the parental species, F_1 and backcross hybrids, and a test on the genetic hypothesis of additivity; (2) an analysis of the principal components of FT diversity, after splitting the FT into sub-components; (3) identification and localization of quantitative trait loci (QTL); (4) impact of QTL on other traits. We subsequently discuss the implications with respect to breeding.

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[1] Caffeine and chlorogenic acids contribute s to bitterness (Leloup et al. 1995)

Table 1 Fructification time (means)[a] for *C. pseudozanguebariae* (PSE) *C. liberica* var. *dewevrei* (DEW), F_1 hybrids and backcross hybrids (BCPSE and BCDEW)

	PSE[b]	BCPSE	F_1	BCDEW	DEW
Fructification time (days)	66[a] (64–70)	134[b] (96–184)	182[c] (171–191)	248[d] (205–291)	314[e] (289–336)

[a] Ranges in Parenthesis
Values followed by different letters are highly significantly different at $P < 0.001$

Materials and methods

Plant material

The plant material included parental species PSE and DEW, the F_1 hybrids and the first backcross generation (BC_1). PSE, DEW, F_1 and BC_1 were planted separately in five plots at the Institut de Recherche pour le Développement (IRD) Coffee Breeding Station of Man, (Ivory Coast.) Planting density was 2.5 × 1.25 m.

PSE is a wild diploid coffee species native to Kenya and Tanzania (Berthaud et al. 1980; Hamon et al. 1984), whereas DEW is a diploid species endemic to the Central African Republic (Dublin 1962, 1963; Berthaud and Guillaumet 1978). The F_1 hybrids arose from a cross between PSE tree no. 8044, used as female, and DEW tree no. 5851. The BC_1 hybrids included ten F_1 × PSE hybrids (BCPSE) and 62 F_1 × DEW hybrids (BCDEW). Backcross hybrids were from a multi-parental origin (male parent DEW not accurately identified).

Dependent on the species and year, blooming occurs between December and March 1 week after a 10 mm rainfall. Fruits (berries) were harvested from April-May for PSE to December for DEW.

Notations

Fructification time corresponds to the time between flowering and fruit ripeness and was recorded every week after the flowering date. It can be separated into three visibly distinct steps: (1) a stationary step (STS), beginning at the flowering date and ending when fruit growth can be visually detected; (2) a growth step (GRS), during which fruit reaches a maximal size; (3) a maturation step (MS), during which green fruit becomes yellow, orange and red. Our analyses focused on 35 BCDEW hybrids.

For seed weight, berries were harvested at complete maturity and depulped using the wet processing method. The seeds were then stored for 24 h at 110 °C in a drying oven. The trait measure was 100-seed weight (W100). Three samples per genotype were evaluated.

Data on caffeine content were obtained by Barre et al. (1998), whereas those on chrorogenic acid (CGA), trigonelline and sucrose contents were recorded by Ky et al. (1999, 2000a, 2001). All experimental methods are described in the cited papers.

Molecular data were obtained by Barre (restriction fragment length polymorphism, RFLP markers) and Ky (amplified fragment length polymorphism, AFLP markers) and led to a published genetic map (Ky et al. 2000b). Only markers present in all PSE trees and absent in DEW were recorded for mapping because of the multi-parental origin of the BCDEW hybrids.

Statistical analysis

To test the additive model for FT quantitative inheritance, we randomly sampled ten trees in each group – PSE, BCPSE, F_1, BCDEW and DEW – resulting in five respective means. A linear regression was then computed between these means and the expected part of the DEW genome in each group: 0% in PSE, 25% in BCPSE, 50% in F_1, 75% in BCDEW and 100% in DEW.

All between-group comparisons were carried out using a one-way ANOVA with fixed factorial effects. When the ANOVA test was significant, a Newman and Keuls test allowed us to finalize the multiple mean comparison (only when there were more than two groups). All statistical analyses were performed using the STATISTICA software package, version 5.1 (1997).

A principal component analysis (PCA; Varimax normalized) was applied to the three visual steps – STS, GRS and MS – in order to verify their dependence or independence.

Lastly, mapping was carried out using the MAPDISTO 1.2.0.3 software package (2002) (Lorieux; http://www.mpl.ird.fr/mapdisto/)

Results

Fructification time in parental species, F_1 and backcross hybrids and the additivity hypothesis

All analyses concerning group characterization and mean comparisons were carried out with a balanced design (ten trees per group) and a one-way ANOVA.

Highly significant differences were noted between PSE, BCPSE, F_1, BCDEW and DEW groups for fructification time ($F_{4, 45} = 231$; $P<0.001$). The between-group effect contributed to 95.8% of the variance observed between the 50 analyzed trees. Fructification time of PSE was 66 days on average, ranging from 64 to 70 days between trees (Table 1). In contrast, in DEW, fructification time required 289–336 days, with a mean of 314 days. In the F_1 hybrids, fructification lasted 182 days on average (Table 1), i.e. close to the theoretical mean parental value (PSE + DEW)/2 = 190 days. Fructification times were 134 and 248 days in BCPSE and BCDEW, respectively, i.e. also close to the expected values (DEW+3*PSE)/4 = 128 days and (3*DEW+PSE)/4 = 252 days.

The additivity test involving PSE, F_1 × PSE, F_1, F_1 × DEW and DEW group means was very highly significant (r=0.998, P<0.0001), with a linear relationship (Fig. 1).

Fructification time components in BCDEW

The STS component of fructification time was 60 days on average (range: 47–82 days), representing 23% of FT; GRS was 112 days on average (range: 76–151 days), i.e. 43% of FT, while the maturation step lasted 87 days on average (61–133 days).

Using a PCA, two principal components explained STS, GRS and MS variations. The first component was characterized by STS (r = 0.836) and GRS (r = 0.832) and differentiated trees with a long STS+GRS phase, e.g. hybrid no. 1719 (209 days), from trees with a short STS+GRS phase (no. 0903; 130 days). The second component represented the maturation time (r = 0.998). PCA analysis structured the fructification time into two

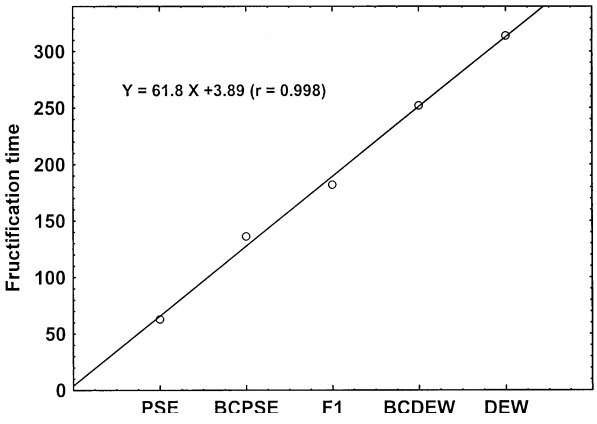

Fig. 1 Additive model for fructification time in the *Coffea pseudozanguebariae* (*PSE*) × *C. liberica* var. *dewevrei* (*DEW*) interspecific cross

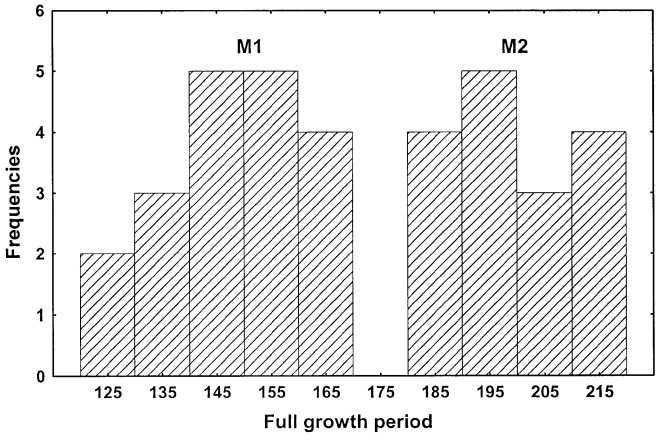

Fig. 2 Bimodal distribution of the full growth period (STS+GRS) in the BCDEW backcross

independent periods: the full-growth period (FGRP), including STS and GRS, and the maturation period. FGRP showed a bimodal distribution with a 19:16 ratio (Fig. 2). The first mode (M1) ranged from 130 to 169 days, with a mean of 149.5 days, whereas the second one (M2) ranged from 184 to 219 days, with a mean of 199 days. The presence of two modes suggests the existence of a major gene, which we call *Ft1*, with two alleles, *Ft1-P* (from PSE) and *Ft1-D* (from DEW).

Fructification time was 235.8 days (191–291) and 287.3 days (262–317) in hybrids belonging to the M1 and M2 groups, respectively. M1 hybrids thus had a higher FT than expected in F_1 (236 days vs. 190 days), whereas M2 hybrids had a lower FT than observed in DEW (287 days vs. 314 days). In addition, the minimum value observed among M1 hybrids was 191 days, close to that of the F_1, whereas the maximum value was 317 days in M2 hybrids, i.e. close to that of DEW.

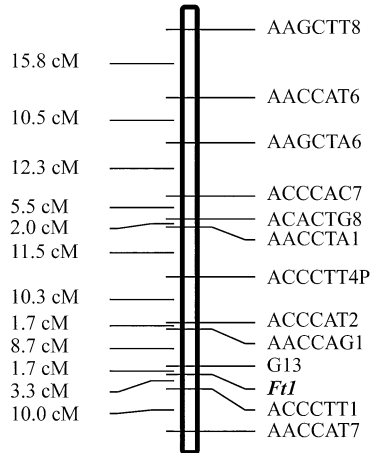

Fig. 3 Linkage group E showing the location of the *Ft1* major gene of fructification time, bracketed by molecular markers G13 and ACCCTT1

Full-growth period and maturation step in parental species

In DEW, FGRP was 214.7 days, representing about 68% of FT. The range was 203–227 days, i.e. a variation of 24 days within species (11% of mean). In contrast, the maturation step was more variable (66–126 days between trees within DEW).

In PSE, the FGRP was 46.5 days, but this represented a similar share of the fructification time (70%). It ranged from 43 to 49 days, i.e. a variation of 6 days within species. Here again the maturation step diversity was higher (15–28 days).

Identification of a QTL and colocalizati on with a major gene

Only one QTL was identified for fructification time using a one-way ANOVA with a significance level of $P<0.001$. The QTL was located on linkage group E defined by Ky et al. (2000), between the AFLP marker ACCCTT1 ($F_{1, 32} = 33$; $P = 0.000006$) and the RFLP marker G13 ($F_{1, 27} = 30$; $P = 0.000009$). The ACCCTT1 marker explained 64% of the FT variance and the mean FT was 285.6 and 237.5 days for genotypes D_1D_1 and D_1P_1, respectively.

Genotypes D_1D_1 and D_1P_1 differed in terms of their full-growth phase ($F_{1,32} = 68$; $P < 0.000001$), with 196.4 and 150.7 days, respectively. In contrast, they were similar with respect to their maturation period ($F_{1,32} = 0.13$; $P = 0.72$). We defined a new Bernoulli variable coded 0 and 1 when FGRP belonged to M2 or M1, respectively, and mapped this variable. This phenotypic marker corresponds to the major *Ft1* gene and was located, as expected, between G17 and ACCCTT1 (Fig. 3) – i.e. colocated with the QTL.

Table 2 Differences and similarities between genotypes D1P1 and D1D1 (marker ACCCTT1) for chlorogenic acid (CGA), caffeine, trigonelline and sucrose contents, and for 100-seed weight. Contents are expressed as an a percentage dry matter basis. In all ANOVA, *df* = 1

	D1P1	D1D1	*df*2	*F*	*P*
CGA	5.67	5.87	51	0.81	0.37
Caffeine	0.48	0.61	55	6.18	0.016
Trigonelline	0.97	0.97	47	0.006	0.94
Sucrose	5.98	6.02	47	0.015	0.90
100-seed weight	9.1	10.3	55	5.83	0.019

Impact on other traits

This analysis concerned a technological trait – seed weight – and four biochemical traits involved in cup quality for which evaluations had been previously carried out and the assumption made that they could be influenced by fructification time through an accumulation process, i.e. chlorogenic acid, caffeine, trigonelline and sucrose contents. Only two traits were significantly related to ACCCTT1: caffeine content and 100-seed weight (Table 2).

The presence of an allele from PSE in genotypes D1P1 led to a 20% drop in the caffeine content with regards to D1D1. Unfortunately, the shortest fructification time due to this allele also led to a 12% decrease in the 100-seed weight.

Discussion

Breeding for a lower caffeine content is a major aim of cup quality improvement in *C. canephora*. To date, there has been no clear interest in studying the genetics of fructification time and its relationship with caffeine content and seed weight. This has largely been due to the low fructification time range within cultivated species relative to the within-genus range. In contrast, the within-genus relationship between fructification time, caffeine content and seed weight opens new possibilities for the genetics of fructification time. From a genetics viewpoint, studying a cross between very different species is a better way to analyze quantitative inheritance of fructification time, caffeine and seed weight.

A major gene for fructification time

Our main result was in identifying and locating, on a genetic map, a major gene (*Ft1*) showing a 1:1 segregation in BCDEW. Most fructification time variations between PSE and DEW could be explained by this gene while highlighting the impact through the growth period. The fructification time quantitative additivity noted is in accordance with the co-dominance effect of *Ft1-P* and *Ft1-D* alleles in the hybrid. This suggests an *Ft1-P* allele effect of reducing fructification by 50 days. The expected value in hybrids that are homozygous (*PP*) at the *Ft1* locus and have 100% of the DEW background genome would be 214 days.

The fact that no QTLs were identified for the maturation phases seems to confirm an independence regarding the full-growth period and the importance of environmental effects. It seems that there were genetic effects since the maturation time was fivefold longer in DEW than in PSE (100 days vs. 20 days). By contrast, the relative part of this phase in FT was constant (30%) in the DEW, PSE, F_1, BCPSE and BCDEW hybrids. If we assume that maturation time is proportional to FGRP, independence and proportionality are paradoxical. A more in-depth analysis suggests that this is a pseudo-paradox since independence was estimated within groups, whereas proportionality was between groups. Taking within-species variations for the maturation step and the multi-parental origin of BCDEW into account, the absence of QTL identification by specific PSE AFLP markers was expected. With our experimental design, we can only identify QTLs with a bearing on between-species differences.

Impact on other traits

The indirect effect of *Ft1* on caffeine content is of particular interest. There was a positive relationship between FGRP and caffeine content. Two hypothesis can be put forward to explain this: (1) there is a linkage between *Ft1* and a locus involved in caffeine content variations; (2) the caffeine content could be the result of a cumulative process occurring in green beans. Further investigations are required to verify these hypothesis by decomposing caffeine content (CAF) into potential components, such as CAF/FGRP, and screening for corresponding QTLs. There was a caffeine decrease of 20% in heterozygote hybrids due to *Ft1*, suggesting that DEW, with two introgressed *Ft1-P* alleles, would theoretically have a 40% lower caffeine content, i.e. 0.6% dmb, instead the 1% dmb currently recorded in DEW.

Introgression of *Ft1-P* alleles in DEW would reduce the seed weight, an important economic trait. A decrease of 12% in the heterozygote (*DP*) suggests a 24% reduction after full introgression. Contrary to caffeine, there was clear dry matter accumulation during FGRP, and the seed weight/FGRP ratio should now be computed to identify other QTLs for seed weight.

The fact that there was no impact of *Ft1* on CGA, trigonelline and sucrose contents, as for CAF, suggests that accumulation could occur during the maturation time or that their content is constant during the early to later maturation stages. With respect to sucrose, its final concentration results from a linear accumulation process in *C. arabica* and *C. canephora* (Rogers et al.1999) but with a between-species difference: in *C. arabica*, accumulation starts as soon as the FGRP, whereas in *C. canephora*, it occurs during the maturation phase. With respect to PSE and DEW, no data are available, but the

absence of impact suggests that the patterns are similar to those of *C. canephora*. There are no published data on trigonelline content variations during fructification. Nevertheless, a QTL for trigonelline content has been located on linkage group G (Ky et al. 2001), whereas no QTLs have been detected for the trigonelline content/fructification time ratio. In addition, quantitative genetics results imply a nucleo-cytoplasmic interaction for this trait (Ky et al. 2001).

Potential for breeding programs

The identification and location of a major gene *Ft1* involved in fructification time in the (PSE × DEW) × DEW interspecific cross could have consequences on cultivated *C. canephora* breeding programs striving to achieve a lower caffeine content. Nevertheless, marked drops in FT would have an important negative impact on seed weight, suggesting that it would be more suitable to use a species such as *C. eugenioides* (FT = 6 months), instead of PSE. Indeed, a 1- to 2-month modification in fructification time would be sufficient to adjust the harvest to the end of the rainy season, thus avoiding moisture during drying. Nevertheless, transferring the *Ft1* major gene from *C. eugenioides* to *C. canephora* should have a limited impact on caffeine content. New research on QTLs for daily caffeine flow should thus be undertaken.

Irrespective of the future use of *Ft1* – conservation or modification of the 10-month fructification time during the introgression process – the identification of molecular markers close to the locus would make it possible to apply early molecular-assisted selection (MAS), which is a powerful technique for perennial plants with a long generation time.

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